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Monitoring tylosin and sulfamethazine in a tile-drained agricultural watershed using polar organic chemical integrative sampler (POCIS)

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HIGHLIGHTS

- Tylosin and sulfamethazine were detected in 37 to 100% of samples at four locations.
- Time weighted antibiotic concentrations were less than 2 ng L^{-1} and were markedly less than the atrazine concentration.
- Direct sampling of the subsurface drainage water showed that antibiotics are leaching through the soil profile.

GRAPHICAL ABSTRACT

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ABSTRACT

This study evaluated the influence of temporal variation on the occurrence, fate, and transport of tylosin (TYL) and sulfamethazine (SMZ); antibiotics commonly used in swine production. Atrazine (ATZ) was used as a reference analyte to indicate the agricultural origin of the antibiotics. We also assessed the impact of season and hydrology on antibiotic concentrations. A reconnaissance study of the South Fork watershed of the Iowa River (SFIR), was conducted from 2013 to 2015. Tile drain effluent and surface water were monitored using polar organic integrative sampler (POCIS) technology. Approximately 169 animal feeding operations (AFOs) exist in SFIR, with 153 of them being swine facilities. All analytes were detected, and detection frequencies ranged from 69 to 100% showing the persistence in the watershed. Antibiotics were detected at a higher frequency using POCIS compared to grab samples. We observed statistically significant seasonal trends for SMZ and ATZ concentrations during growing and harvest seasons. Time weighted average (TWA) concentrations quantified from the POCIS were 1.87 ng L^{-1} (SMZ), 0.30 ng L^{-1} (TYL), and 754.2 ng L^{-1} (ATZ) in the watershed. SMZ and TYL concentrations were lower than the minimum inhibitory concentrations (MIC) for *E. coli*. All analytes were detected in tile drain effluent, confirming tile drainage as a pathway for antibiotic transport. Our results identify the episodic occurrence of antibiotics, and highlights the importance identifying seasonal fate and occurrence of these analytes.

1. Introduction

Antibiotics have been used in livestock production since the early 1950's for growth promotion (subtherapeutic), disease prevention

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(prophylactic), and disease treatment (therapeutic use). In 2013, the total dispersal of approved antibiotics for food producing livestock was approximately 14.9 million kilograms, in which 99.3% of that total dispersal was used, domestically in the United States (FDA, 2015). In a five-year span between 2009 and 2013, the domestic sale and distribution of antibiotic active ingredients for agricultural use increased approximately 17%, while those classified as medically important increased 20% (FDA, 2015).

Subtherapeutic use of antibiotics in animal feed and water for growth promotion is a concern due to their ability to select resistant bacteria in the gastrointestinal tract of livestock (Chee-Sanford et al., 2009). These antibiotics are not fully metabolized in livestock and are excreted as the parent compound or as a metabolite (Kim et al., 2011; Joy et al., 2013; Kemper, 2008). Antibiotics enter the environment via land application of manure or lagoon treated water (Kim and Carlson, 2007). Once delivered into the terrestrial environment, their potential to induce antibiotic resistance is a cause for concern. Recently, the U.S Federal Drug Administration (FDA) introduced a strategy to combat antibiotic resistance, with the issuance of "Guidance for Industry" (GFI) documents #209 (FDA, 2012) and #213 (FDA, 2013) and the Veterinary Feed Directive (VFD). The VFD requires the supervision of a licensed veterinarian for the use of drugs in or on animal feed. Currently, all antibiotics ranked under GFI #152 (FDA, 2003) are classified as medically important to human health, and include the macrolide antibiotic tylosin and the sulfonamide antibiotic sulfamethazine.

To investigate the potential relationship between antibiotic resistance and low environmental concentrations, monitoring strategies are needed to detect these low concentrations. Pruden et al. (2013) suggests that strategic monitoring is needed to provide baseline data on antibiotics, residues, and antibiotic resistance genes (ARGs). Since the first national reconnaissance pharmaceutical water quality study (Kolpin et al., 2002) the investigation of the occurrence, fate, and transport of emerging contaminants has become more prevalent. From this study and others, antibiotics have been detected in surface water (Fairbairn et al., 2015; Ou et al., 2015; Gao et al., 2012), ground water (Barber et al., 2008; Campagnolo et al., 2002; Watanabe et al., 2010), soil (Joy et al., 2013; Kurwadkar et al., 2011), sediment (Gao et al., 2012; Ok et al., 2011; Kim and Carlson, 2007), and crops (Carter et al., 2014; Bassil et al., 2013; Wu et al., 2011; Jones-Lepp et al., 2012; Dolliver et al., 2007).

Water quality monitoring of antibiotics and other emerging contaminants is difficult due to their diverse physiochemical properties and their interactions in the environment. Traditional environmental sampling techniques including discrete grab samples and automatic samplers have been used for emerging contaminants. These sampling techniques often require extracting large volumes of water to detect these contaminants (Söderström et al., 2009 and Alvarez et al., 2005). The greatest shortcoming of discrete grab sampling, is that it only provides a snapshot of environmental levels, neglecting episodic events and overestimating concentrations. The use of these sampling methods can be expensive and time-consuming (Söderström et al., 2009; Alvarez et al., 2007). The development of passive sampler technology such as the Polar Organic Chemical Integrative Samplers (POCIS) has potentially provided a better alternative for sampling polar organic contaminants such as tylosin, sulfamethazine, and atrazine.

The POCIS is a dynamic monitoring tool, which has the ability to detect ultra-low concentrations of the dissolved phase of chemicals. The POCIS has three general designated uses: screening of pollutants, determination of TWA concentrations, and toxicity bioassay analysis. The screening capability of the POCIS allows for the determination of the source and concentration gradient of chemicals. The application of screening and TWA determination allows for the evaluation of spatial and temporal distribution in aquatic environments (Morin et al., 2012; Söderström et al., 2009). The ability of the POCIS to screen pollutants was also shown in a study conducted by Kolpin et al. (2013) where POCIS were used to determine the exposure of chemical contaminants to smallmouth bass in the Potomac River basin. Among the chemical contaminants tylosin, sulfamethazine, and atrazine detection frequencies were 0, 40, and 100% respectively. Recently, Jaimes-Correa et al. (2015) used the POCIS to determine the seasonal occurrence of 12 different antibiotics, including tylosin and sulfamethazine, and a beta agonist in a predominantly agricultural watershed in Nebraska. The tylosin and sulfamethazine did not show any spatial or temporal variation in that watershed. Morin et al. (2012) has noted the application of the POCIS to the detection and quantification of an estimated 300 chemicals. The POCIS is an extensive tool that has been used in many aquatic environments including: rivers, streams, creeks, estuaries, lakes, seas, bays, and harbors.

We conducted a reconnaissance study of the SFIR, to establish the baseline water quality levels in respect to sulfamethazine (SMZ) and tylosin (TYL), and determine their distribution in the watershed using POCIS technology. Our objectives were to investigate the influence of temporal and spatial variation on the occurrence, fate, and transport of tylosin and sulfamethazine; determine the frequency of detection, and assess the impact of tile drainage vs. surface water on antibiotic loads and concentrations. Tylosin and sulfamethazine were chosen because they are used in swine production and we had previously detected tylosin in agricultural drainage water (Garder et al., 2014). Atrazine was included as a reference compound as it has often been detected in agricultural watersheds.

2. Materials & methods

2.1. Watershed description

The South Fork watershed (SFIR) is a predominantly agricultural watershed, which encompasses approximately 78,000 ha (193,000 acres). The greater part of SFIR is located in Hamilton and Hardin counties in north central Iowa, with the most northern part located in Wright and Franklin counties. Three major drainage areas make up the SFIR; Tipton Creek tributary in the southwest, South Fork of the Iowa River in the center, and the Beaver Creek tributary in the southeast. The headwaters of the South Fork of the Iowa River originate from three subsurface drains located in Hamilton County. From the headwaters, the South Fork flows in a northeasterly direction until entering Hardin County where it flows in a southeasterly direction meeting the Iowa river south of Eldora (McCarthy et al., 2012).

The SFIR is dominated by agricultural land covering approximately 96% of the watershed. There is a large concentration of animal production facilities along with intense row cropping. There are approximately 169 animal feeding operations (AFOs) in the watershed, with 153 of them being swine facilities (Fig. 1), accounting for 91% of AFOs. Swine seem to have a higher frequency of bacteria with antibiotic resistant genes (ARG), which directly correlates with the amount of antibiotics used by the swine industry compared to cattle or sheep (Heuer et al., 2011). Swine manure produced from treated pigs, has been shown to enhance the spread of antibiotic resistance in soil bacterial communities (Heuer et al., 2011). Campagnolo et al. (2002) showed that antibiotics are transported from swine farms to proximal surface and ground water. The prevalence of antibiotic resistant bacteria was further documented in swine herds by (Chander et al., 2007; Mathew et al., 2001). According to Tomer et al. (2008a), the estimated swine population of the watershed is 601,193 (Beaver Creek: 75,379, South Fork: 301,628, and Tipton Creek: 224,186). The resulting swine densities are 4.14 (Beaver Creek), 7.9 (South Fork), and 11.29 (Tipton Creek) swine ha^{-1}. More recently Hamilton and Hardin counties were estimated to have a swine inventory of 1.37 million (USDA-NASS, 2012). Previous work shows that the SFIR contains persistent populations of *E coli* and *Enterococcus* (Tomer et al., 2008a), and genes associated with zoonotic pathogens (Givens et al., 2016), suggesting that transport of antibiotics within this watershed is likely. Finally, three small towns with a combined human population of <500 have sewage treatment facilities the

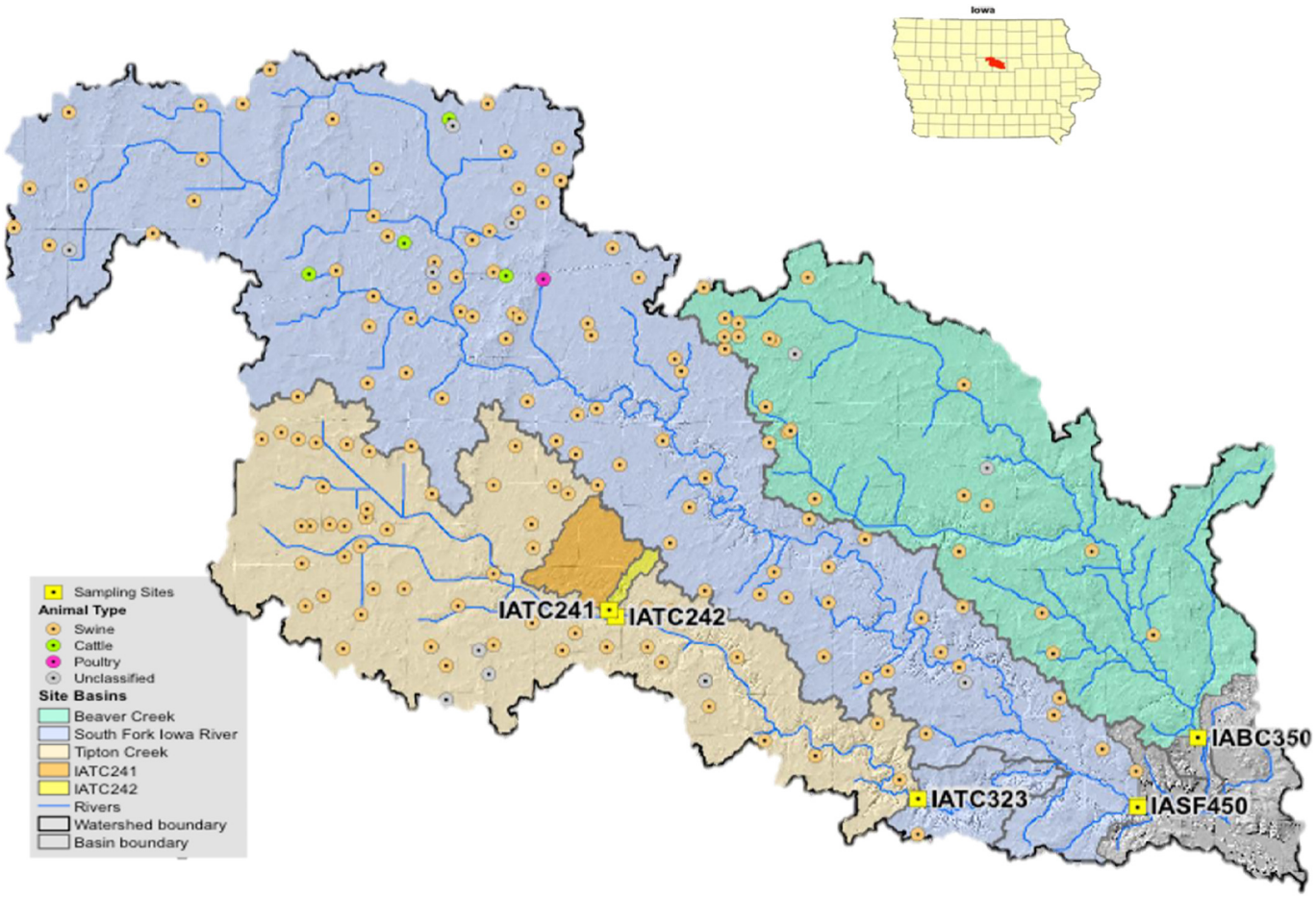

Fig. 1. Animal feeding operations (AFO) and sampling site locations in the South Fork watershed of the Iowa River (SFIR). The AFO's are categorized by swine, cattle, poultry, and unclassified. The map inset shows the SFIR watershed boundary in central Iowa.

discharge within the watershed, with potential use of SMZ and TYL in humans or companion animals.

Historically corn and soybeans are the crops grown in the watershed, and that remains the trend today (Tomer et al., 2008b). Greater than 85% of the agricultural land is used for the production of corn and soybeans, map shown in Supplementary Information (SI Fig. 1). Planting occurs in April to May and harvesting occurs from September to October. The manure produced from the CAFOs (confined animal feeding operations) in the SFIR is the main source of nutrient application. Inorganic fertilizers and a broad band of herbicides are also used for increased crop production (McCarthy et al., 2012).

Approximately 54% of watershed consist of hydric soils (Tomer and James, 2004). These hydric soils include; Clarion, Nicollet, Webster, Harps, and Okoboji soil classifications. Due to these hydric soils, artificial drainage has changed the hydrology of the SFIR. Approximately 80% of the SFIR is tile drained (Green et al., 2006). Vertical surface drains, coupled with subsurface tile drains, route water from the agricultural landscape directly into drainage channels or streams. As a result, the water table is lowered which ensures agricultural lands are ready for cultivation and the root zone is not saturated. Consequently, artificial drainage expedites the transport of dissolved forms of nutrients and chemicals, including agricultural emerging contaminants (AEC), to surface waters, thereby negatively impacting water quality (Frey et al., 2015; Qi et al., 2011; Kay et al., 2004; Campagnolo et al., 2002; Gentry et al., 2000; and Kladivko et al., 1991). Work conducted by Schilling et al. (2012) indicates that tile drainage is a key mechanism impacting fundamental watershed characteristics and should be evaluated when investigating pollutant delivery from agricultural environments.

2.2. Sampling sites

Five field sites in the central to southern part of the SFIR were monitored, including IATC-241, IATC-242, IATC-323 (Tipton Creek tributary); IASF-450 (South Fork tributary); and IABC-350 (Beaver Creek tributary) (Fig. 1). These stations were selected because of the ongoing collection of hydrology and water quality data by USDA-ARS. Sites IATC-241 and 242 are tile drain discharge points, while the other three sites are in-stream stations. The drainage area of the sampling sites is shown in supplementary information (SI Table 1). The mean precipitation at the sampling sites in SFIR was (849.4 $\pm$ 104.4 mm year^{-1}), increasing from 2013 to 2015.

2.3. Water samples

USDA-ARS operates tipping bucket rain gauges (Texas Electronics TE525), high-accuracy stage recordings (PS-2 pressure sensor and high-accuracy stage OTT CBS bubbler recorder), thermocouples for air and stream temperature (Type-T thermocouple), flow meters (WaterLog H-355 bubbler), Teledyne ISCO 6712 samplers, and data loggers (Campbell Scientific CR1000) at each sampling site. Samples were collected in 2013, 2014, and 2015 from April to November, to include planting, growing, and harvest seasons for corn and soybean. Sampling frequency was initially monthly, but was increased to bi-monthly in 2014 and 2015, to capture more episodic events.

To monitor the AEC concentrations in water, duplicate grab samples were collected from the tile outlets and in streams at the corresponding sites. Grab samples were collected in 0.5 L amber glass jars with PTFE-

lined caps. Grab samples were kept on ice in the field and stored at 4 °C, at USDA-ARS NLAE (National Laboratory of Agriculture and Environment) prior to analysis. Tiles maintained flow throughout the majority of the sampling season.

All POCIS (Environmental Sampling Technologies Laboratory) were stored frozen prior to use, thawed and preconditioned in deionized water for 24 h, and transferred to the field in sealed cans until their deployment. POCIS were deployed at four of the five sampling sites, (IATC-241, IATC-323, IASF-450, and IABC-350). Due to the elevated height of the (IATC-242) tile drain outlet, a POCIS sampler couldn't be successfully installed and submerged in the flow path of the tile discharge, and thus only grab samples were collected at this site. To protect the POCIS during deployment, they were housed in stainless steel perforated protective canisters (Alvarez, 2010). Depending on the location and physical characteristics of the site, the POCIS canisters were mounted or suspended in the waterbody, and anchored with wire cable to the shore. Due to the high flow at IATC-241, the POCIS canister was located to the side of the tile drain to prevent POCIS from being punctured by high-velocity flows and debris.

2.4. Sample analysis

2.4.1. Extraction procedure

POCIS extraction procedure was adapted from the protocols used by Alvarez et al. (2004) and Mazzella et al. (2007). POCIS was disassembled and hydrophilic-lipophilic balance (HLB) sorbent material was washed with 20 mL of acetonitrile-isopropyl alcohol (50:50, v:v) into a 60 mL SPE reservoir, fitted with a 20 μm frit. A second 20 μm frit was placed on top of the transferred solvent, before elution. The washing solvent was collected and then combined with 100 mL of acetonitrile-isopropyl alcohol to elute the sorbent material. The washing solvent was not discarded because testing showed significant amounts of constituents were found in the solution. Once the 120 mL of solvent was eluted, 250 μL of simetone dissolved in MeOH was added at a concentration of 42 ng mL^{-1} as an internal standard. The combined extract and wash was then evaporated down to 0.2–0.3 mL using a nitrogen evaporator. After evaporation the residual solvent was reconstituted to 2 mL using 10 mM ammonium acetate and allowed to reach equilibrium for approximately 30 min. After equilibrium, samples were filtered using a 13 mm 0.2 μm pore nylon syringe filter and submitted for analysis.

In addition, POCIS residues from the SPE reservoirs were placed in 100 mL beakers and filled with 60 mL of solvent. Each residue sample soaked for 24 h, extract and wash were collected, and 125 μl of internal standard was added. Extract and wash were evaporated down to 0.2 mL, reconstituted with ammonium acetate to 2 mL and submitted for analysis. The POCIS + POCIS residue concentrations were summed after analysis, providing the total mass concentration accumulated on the POCIS. A lab spike (5 ng L^{-1} of each analyte) and lab blank (deionized water) were processed with each set of POCIS samples. The spike was used to determine POCIS extraction efficiency. POCIS extraction yielded 108% (ATZ), 82% (SMZ), and 81% (TYL) extraction efficiencies.

Grab samples were first processed by filtering 250 mL of sample through 0.45 μm filter, eliminating particulate matter. Oasis HLB cartridges were preconditioned with 2 mL of MeOH, and drawn down, followed by 2 mL of Milli-Q water. Samples were then eluted through Oasis HLB solid phase extraction (SPE) cartridges with acetonitrile-isopropyl alcohol. Simetone was also used as an internal standard for the grab samples as described previously and extracts were evaporated down, reconstituted, filtered, and submitted for analysis.

2.4.2. AEC analysis

Analysis was performed using an ABSciex 5500 QTrap mass spectrometer with an Agilent 1260 Infinity LC. Separation took place on a Phenomenex-Gemini - 3 μm C18 110 A column, 50 × 2.0 mm, at a flow rate of 0.5 ml/min. Mobile phase A was 0.1% formic acid in water and B was 0.1% formic acid in methanol. The LC gradient begins at 98% A and holds for 0.3 min, then ramps to 20% A in 7.27 min, then rapidly increases to 1% A by 7.37 min and is held for 3.53 min. The column is re-equilibrated back to the initial conditions, for a total run time of 15 min. Compounds were monitored using multiple reaction monitoring (MRM), with 3 stages collected for each. The most abundant transition was used for quantitation, and the second and third product ions were used for ion ratio confirmation. Acceptance criteria for the ions were based on the European Standard, which uses a larger acceptance range for smaller ion ratios as follows: the ratio is between 0 and 10% when the acceptable percent difference is 50, if the ratio is 10–20% the acceptable difference is 30%, a ratio range of 20–50% must agree with a percent difference of 25, and a ratio above 50% has an acceptable percent difference of 20 (European Standard EN 1662, 2008). The precursor and product ion masses and optimized mass spectrometer conditions for the determinations of SMZ, TYL, and ATZ are shown in (SI Table 2).

All sample extracts were analyzed for SMZ, TYL and ATZ. The instrumental limit of detection (LOD) and limit of quantification (LOQ) were determined for each analyte for water and POCIS (Table 1). Instrumental LOD and LOQ is the smallest signal above background noise that an instrument can detect or quantify reliably. The LOD and LOQ for POCIS samplers are back-calculated based on the analytical protocol and on the sampling rate, R_s (Poulier et al., 2015).

2.5. POCIS time-weighted average concentrations and calibration

Time-weighted average (TWA) concentrations of river and drainage water were calculated using experimentally determined POCIS uptake rates (R_s, L d^{-1}), sampling duration (t), the analyte mass accumulated (M_s, g), and the concentrations were quantified from POCIS extracts (C_s, ng L^{-1}) by mass spectrometry. The TWA was determined by the following equation:

$$\mathrm{TWA} = \frac{C_s M_s}{R_s t} \tag{1}$$

POCIS uptake rates for each target compound were calculated from lab calibration experiments using the following equation:

$$R_s = \frac{C_i - C_t}{C_i} \times \frac{V_T}{t} \tag{2}$$

where, (C_i and C_t, ng L^{-1}), initial concentration and concentration at time, t. V_T is the total volume of water at the time of calibration.

R_s values were determined by using a static depletion laboratory calibration method (Morin et al., 2012). Duplicate two-liter solutions containing ATZ, SMZ, and TYL at 60 ng mL^{-1}, were prepared and a single

Table 1
Instrumental and matrix limits of detection (LOD) and limit of quantification (LOQ) for atrazine (ATZ), sulfamethazine (SMZ) and tylosin A (TYL).

Instrumental			Grabs		POCIS	
Analyte	Limit of detection (ng L^{-1})	Limit of quantification (ng L^{-1})	Limit of detection (ng L^{-1})	Limit of quantification (ng L^{-1})	Limit of detection (ng L^{-1})	Limit of quantification (ng L^{-1})
SMZ	0.041	0.04	0.000328	0.00032	0.00016270	0.000159
TYL	0.044	0.04	0.000352	0.00035	0.00000965	0.000009
ATZ	0.027	0.03	0.000216	0.00024	0.00009574	0.000106

POCIS was added to each container. Negative and positive controls were also prepared. The negative control consisted of ultra-pure water with a POCIS, whereas the positive control was spiked ultra-pure water with ATZ, SMZ, TYL at 60 ng mL^{-1}. The positive control accounted for the natural degradation of the analytes. Duplicate water samples were taken each day for 21 days and the solution concentrations were determined as described previously. To protect against photodegradation and evaporation, the calibration experiment was conducted in the dark and each vessel was fully covered. Sampling rates (R_s) were quantified for SMZ (0.084 L d^{-1}), TYL (1.52 L d^{-1}), and ATZ (0.094 L d^{-1}), respectively.

The laboratory sampling rates calculated in this study are different than what the literature reports. In general, it is very difficult to compare laboratory R_s values between studies due to the difference in calibration methods, conditions of the calibration system, and calculation methods used in different experiments (Morin et al., 2012). From literature, we know R_s values are influenced by temperature, water flow/turbulence/agitation, biofouling, POCIS configuration, pH, physiochemical properties, conductivity and salinity. The literature reports laboratory R_s for ATZ ranging from 0.240 ± 0.056–0.290 ± 0.003 L d^{-1} (Thomatou et al., 2011; Bartelt-Hunt et al., 2011; MacLeod et al., 2007). In comparison, our R_s is nearly three-fold lower at 0.094 L d^{-1}. But, Alvarez (1999) reported a laboratory R_s of 0.050 L d^{-1} for ATZ, showing the wide variability of these sampling rates. In comparison, literature laboratory R_s values ranged from 0.049 ± 0.040 to 0.243 ± 0.003 L d^{-1} (Bartelt-Hunt et al., 2011; Mazzella et al., 2007) for SMZ, showing our R_s of 0.084 L d^{-1} lies within this range. Lastly, our R_s value for TYL of 1.52 L d^{-1} was close to the literature value of 1.33 ± 0.151 L d^{-1} reported by Bartelt-Hunt et al. (2011). The variability of R_s values in our study compared to others is significant because variability affects the magnitude of TWA concentrations (Eq. (1)), but the relative differences in TWA concentrations between samples within this study would not be affected. Thus, R_s values are semi-quantitative and not truly quantitative.

2.6. Statistical analysis

Due to the number of samples with non-detectable concentrations, SMZ ($n = 70$ of 290) and TYL ($n = 136$ of 290) assumptions of normality are not met and data are considered censored. Censored observations (non-detects) are defined as low-level concentrations that measure between 0 and the detection/reporting limit of laboratory analytical equipment (Heisel, 2012). Tobit censored regression analysis was used to account for censoring of the dependent variable, y, where y is the analyte concentration, such that y = site + season + year. These measurements are considered imprecise and are commonly reported as an analytical threshold, less than some value. The detection limit for each analyte was back-calculated, removing the less than notation and then input into the Tobit model, acting as a threshold limit for the censored observations in each data set. The Tobit model was used to determine differences in analyte concentration, based on site, season, and year. Seasons were defined as: Pre-Planting (March–May); Growing (June–August); and Harvest (September–November). Pearson product-moment correlation coefficient was determined for each analyte model. Additionally, interactions between site, season, and year were analyzed. Significant differences for all comparisons were evaluated at $p < 0.05$. Statistical analysis was performed using SAS 9.4.

3. Results & discussion

3.1. Occurrence of AECs

POCIS TWA concentrations were determined for four sampling sites in the SFIR watershed from May–November (2013), April–November (2014), and March–November (2015). TYL, SMZ, and ATZ were detected at all sampling sites in every year. Detailed seasonal occurrence and concentration data for each sampling site is provided in (Table 2). From 2013 to 2015, the detection frequencies for SMZ and TYL were 83% and 70%, respectively. ATZ, which is ubiquitous throughout the Midwest (Van Metre et al., 2017; Kolpin et al., 2010; Battaglin et al., 2005), was detected in 100% of the samples in the SFIR watershed. The detection rates of these analytes are comparable to other studies using POCIS samplers in agricultural settings (Table 3). Jaimes-Correa et al. (2015) reported concentrations of SMZ fairly close to those observed in the SFIR, while TYL was an order of magnitude lower than SFIR concentrations. The detailed annual and seasonal occurrence of each analyte is available in Supplemental Information (SI Table 3).

Table 2
Summary of seasonal mean, median, and maximum POCIS analyte concentrations in SFIR from 2013 to 2015, based on sampling site.

Site	Analyte	Season	Mean (ng L^{-1})	Median (ng L^{-1})	Max (ng L^{-1})	% Non-detects
IATC-241	SMZ	Preplant	0.92	0.74	2.10	0.0
		Growing	6.54	1.95	44.08	0.0
		Harvest	1.83	0.87	14.12	3.7
	TYL	Preplant	0.18	0.02	1.51	30.0
		Growing	0.07	0.03	0.27	11.8
		Harvest	0.18	0.03	0.77	57.7
	ATZ	Preplant	368	128	1,949	0.0
		Growing	288	239	939	0.0
		Harvest	128	115	458	0.0
IATC-323	SMZ	Preplant	0.6	0.6	1.2	10.5
		Growing	6.2	0.9	32.7	12.0
		Harvest	1.0	0.7	2.8	31.0
	TYL	Preplant	0.19	0.03	1.46	44.4
		Growing	0.87	0.10	6.92	48.0
		Harvest	0.23	0.06	1.33	48.3
	ATZ	Preplant	1,704	458	15,357	0.0
		Growing	882	387	4,880	0.0
		Harvest	146	122	538	0.0
IABC-350	SMZ	Preplant	0.29	0.24	0.52	62.5
		Growing	0.72	0.40	1.99	45.5
		Harvest	0.39	0.28	1.00	48.3
	TYL	Preplant	0.21	0.03	0.89	57.9
		Growing	0.10	0.06	0.61	43.5
		Harvest	0.19	0.07	1.00	58.6
	ATZ	Preplant	2,152	563	10,817	0.0
		Growing	983	452	4956	0.0
		Harvest	102	91	386	0.0
IASF-450	SMZ	Preplant	0.41	0.35	0.65	36.8
		Growing	5.30	0.70	66.92	8.0
		Harvest	1.08	0.50	6.46	25.9
	TYL	Preplant	0.24	0.04	1.46	47.4
		Growing	3.45	0.08	22.80	52.0
		Harvest	0.75	0.07	2.35	66.7
	ATZ	Preplant	1,968	789	13,041	0.0
		Growing	1,054	354	7,518	0.0
		Harvest	170	76	692	0.0

The physicochemical properties of SMZ indicate it is loosely sorbed in the soil matrix, allowing for it to be highly mobile in the aqueous phase (Wegst-Uhrich et al., 2014; Carstens et al., 2013; Boxall et al., 2002). Degradation behavior of SMZ, shows an initial rapid degradation followed by a slowdown period, reducing its dissipation in soil (Lertpaitoonpan et al., 2015). These properties show the ability of SMZ to be relatively persistent in the environment. In each of the sample years, SMZ was detected above 70%; 93% (2013), 72% (2014), and 84% (2015). In comparison, TYL physicochemical properties indicate that it is more likely to be tightly sorbed and degrade very quickly in the soil matrix and not as available for transport (Wegst-Uhrich et al., 2014; Blackwell et al., 2007; Lee et al., 2007). Contrary to the expected retention of TYL in the soil, TYL had a detection frequency of 70% in the SFIR. TYL was persistent throughout the sample seasons with detection frequencies of: 93% (2013), 47% (2014), and 69% (2015).

3.2. Tobit censored regression analysis

From the Tobit model, the sigma parameter measures the estimated standard error of the regression, which is then compared to the

Table 3
Comparison of atrazine (ATZ), sulfamethazine (SMZ) and tylosin (TYL) concentrations in SFIR watershed to concentrations in other agricultural watersheds using POCIS samplers.

Site name	Area	Study duration	Land cover	Analyte	Detection Freq	Mean Conc	Source
The River Trec, France	200 km^2	Apr.–Jun. 2013	corn, wheat, rapeseed, arboriculture, vegetables	ATZ	100%	6–29 ng L^{-1}	Poulier et al., 2014
Auvézére River, France	900 km^2	Jan.–Sept. 2002	agric. lands (73%) grasslands (50%) cereal crops (28%)	ATZ DEA	45–60% 90–100%	6–8 ng L^{-1}	Poulier et al., 2015
Shell Creek Watershed, Nebraska USA	1200 km^2	Sept.–Nov. 2008 Jun.–Oct. 2009	cultivated land cover, 1550 farms (swine, cattle, poultry)	SMZ TYL	94.5%	1.3 ng L^{-1} 0.034 ng L^{-1}	Jaimes-Correa et al., 2015
South Nation Watershed, Canada	3915 km^2	May–Jul. 2010	corn-soybean, tile drainage	ATZ	100%	6–256 ng L^{-1}	Dalton et al., 2014
Yangtze Estuary, China	30,000 km^2	Oct.–Dec. 2013	aquaculture fisheries	SMZ	100%	40.7 ng L^{-1}	Shi et al., 2014
South Fork of the Iowa River, Buckeye Iowa USA	264.7 km^2	Jun.–Aug. 2013	cultivated crops (90.4%), subsurface drainage (88.7%)	ATZ	–	610.4 ng L^{-1}	Van Metre et al., 2017
South Fork of the Iowa River, New Providence, Iowa USA	582.4 km^2	Jun.–Aug. 2013	cultivated crops (85.7%), subsurface drainage (84.8%)	ATZ	–	211.2 ng L^{-1}	Van Metre et al., 2017
South Fork Watershed of the Iowa River, Iowa USA	781 km^2	May–Nov. 2013 Apr.–Nov. 2014 Mar.–Nov. 2015	agric. lands (96%) corn-soybean, tile drainage (80%), 169 AFOs	SMZ TYL ATZ	83% 70% 100%	1.87 ng L^{-1} 0.30 ng L^{-1} 754.2 ng L^{-1}	Current study

standard deviation of the dependent variable, y, indicating if there is statistical significance in the model parameter estimates. Based on the sigma values, the model fit for the Tobit was statistically significant for all analytes (SMZ, TYL, and ATZ) for POCIS and grabs (Table 4). To further quantify model fit, the Pearson product-moment correlation coefficient was estimated for predicted concentrations versus actual concentrations, which results showed statistical significance except for the POCIS tylosin model ($p = 0.7469$). Model fit was further improved for each analyte by including interactions, which were all statistically significant (SI Table 4).

3.3. Temporal variation

3.3.1. POCIS samples

A pattern of temporal variation was observed for SMZ, TYL and ATZ in the SFIR, on an annual and seasonal scale (Fig. 2 and SI Fig. 2). SMZ exhibited significant differences in concentration ($p < 0.05$) between 2013 and 2015. SMZ was significantly higher ($p = 0.0033$) in 2014 with a TWA 2.83 ng L^{-1}, while there was no statistical difference between 2013 and 2015. TWA of TYL, 1.54 ng L^{-1} was significantly higher in 2013 than in the subsequent years of the study. ATZ showed a strong annual variation in 2013 ($p < 0.0001$) and 2014 ($p = 0.0170$), significantly higher in 2013 (2227.9 ng L^{-1}) compared to 2014 (478.6 ng L^{-1}). POCIS monitoring of SMZ and TYL by Jaimes-Correa et al. (2015) did not report significant temporal variation of these antibiotics.

Next, we examined the impact of seasonality. From the results of the Tobit model, a pattern of seasonality was found only for SMZ and ATZ in the SFIR from 2013 to 2015 (Fig. 2). The growing season for SMZ was statistically significant ($p < 0.0001$). Peak SMZ concentrations occurred during this time period and accounted for the highest detection frequency of SMZ, at 92%. There was no significant difference between harvest and preplant concentrations. ATZ seasonality was significant during growing and harvest season, with ($p < 0.0001$) for both. Growing and harvest seasonality could be linked to high base flow conditions. Base flow accounted for 54% of total flow during the growing season and 72% of total flow during harvest season. Base flow was separated from the hydrograph using an algorithm developed by (Arnold and Allen, 1999; Arnold et al., 1995). The seasonal pulses of these veterinary antibiotics that occurred during the growing and harvest season in the SFIR, is consistent with studies that indicate similar patterns of occurrence and detection during summer months (Jaimes-Correa et al., 2015; Kim and Carlson, 2007; Lissemore et al., 2006). High atrazine TWA concentrations occurred predominantly during preplant in the month of May. This relationship was not significant, but it coincides with the period when ATZ is typically expected to be high due to previous heavy use of herbicides and periods of heavy precipitation, resulting in the first flush phenomenon (Thurman et al., 1991; Graziano et al., 2006). Overall, there was a decreasing trend in TWA concentrations for ATZ, from preplant to harvest. The high detection frequency of ATZ throughout this study, is most likely due to its slow degradation and high persistence in the watershed. TYL, did not exhibit a trend of seasonality, which may be due to its tendency to be tightly sorbed and

Table 4
Comparison summary of the Tobit model fit to POCIS or grab sampling based on sigma values.

POCIS samples				Grab samples*		
Analyte	Standard deviation	σ	Sigma *P*-value	Standard deviation	σ	Sigma *P*-value
SMZ	6.417	6.853	<0.0001	0.0031	0.0037	<0.0001
TYL	1.896	2.642	<0.0001	0.0221	0.0302	<0.0001
ATZ	1842.7	1521.8	<0.0001	0.4359	0.3902	<0.0001

* Grab samples include a total of 5 sites, not 4 like the POCIS.

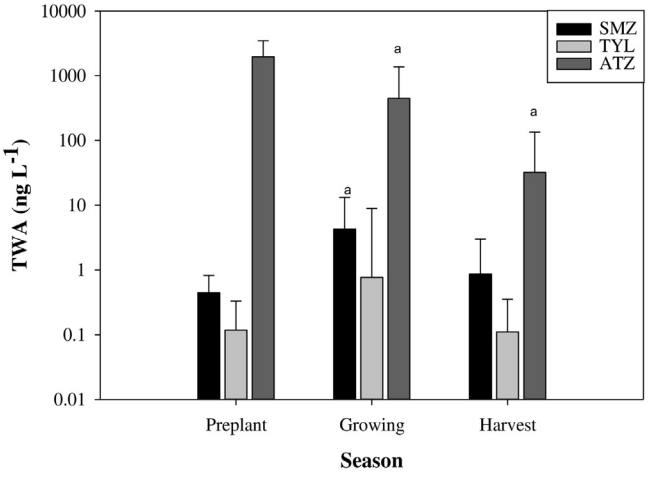

Fig. 2. POCIS time weighted average (TWA) contaminant concentrations across the SFIR watershed, based on season from 2013 to 2015. The error bars indicate the standard deviation and seasonal significance for analyte concentrations ($p < 0.05$) is indicated by the letter (a) above the bar.

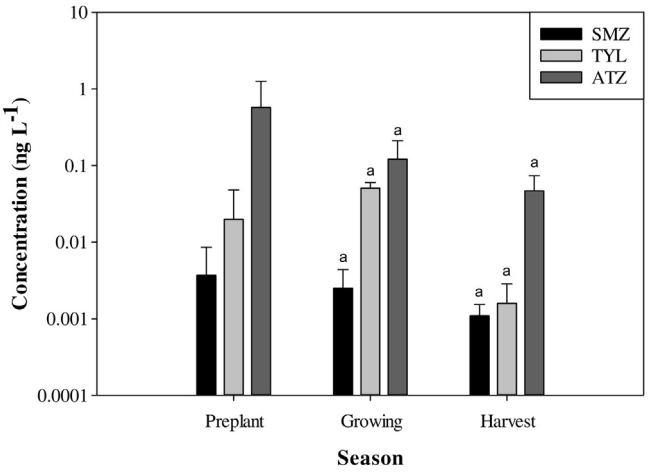

Fig. 3. Grab-sample determined average contaminant concentrations across the SFIR watershed, based on season from 2013 to 2015. The error bars indicate the standard deviation and seasonal significance for analyte concentrations ($p < 0.05$) is indicated by the letter (a) above the bar.

unavailable in the aqueous phase. There was also a decrease in detection of TYL from 2013 to 2015, 93.1% (2013), 47% (2014), and 69% (2015).

3.3.2. Grab samples

A similar seasonal trend that was seen in the POCIS derived concentrations was verified by grab samples (Fig. 3), where growing and harvest seasons were statistically significant in the Tobit model for SMZ, TYL, and ATZ. In addition, concentrations for all analytes were significant in the Tobit model for sampling years 2013 and 2014 for grab samples (SI Fig. 3).

3.4. Impact of tile drainage and hydrology

In this study, site IATC-241 provided the only direct measurement of tile drain effluent using the POCIS sampler. The other three sites monitored all have indirect contributions from tile drain outflows into surface water upstream of those sites, but may also be affected by in-stream processes after drainage enters the stream channel. Tile drain mean TWA concentrations were 3.0 ng L^{-1} (SMZ) and 0.14 ng L^{-1} (TYL), with detection frequencies of 100% and 81%, respectively (Table 2 and Table SI-3). Maximum TWA concentrations were higher for SMZ

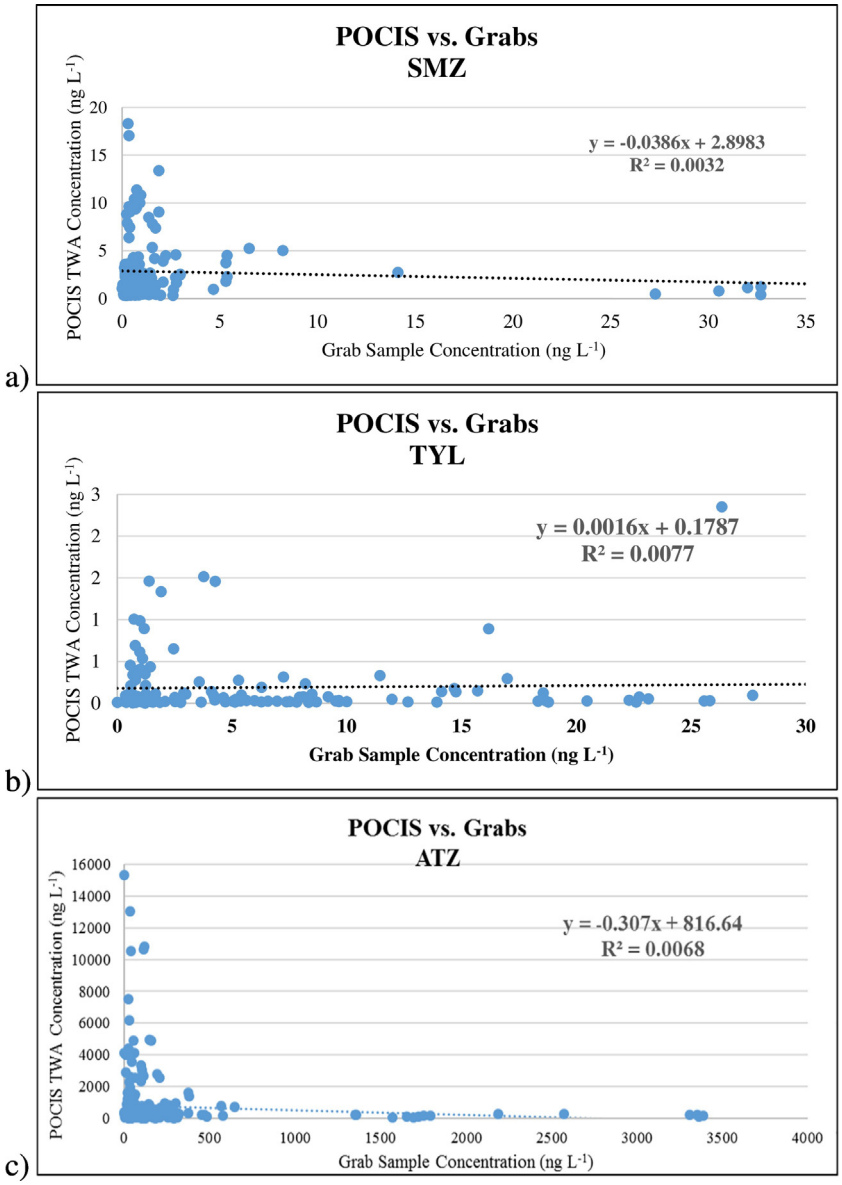

Fig. 4. Comparison of contaminant concentrations obtained by POCIS and grab-sample in the SFIR watershed for (a) sulfamethazine, (b) tylosin, and (c) atrazine.

at 44.1 ng L^{-1} than for TYL at 1.51 ng L^{-1}. SMZ was more prevalent than TYL from the tile drain. In comparison, studies that monitored SMZ and TYL in other Iowa agricultural settings (Cain et al., 2008; Garder et al., 2014), found concentrations an order of magnitude higher than concentrations from IATC-241. The baseflow contribution at IATC-241 was approximately 64% of the total flow from 2013 to 2015. The percentage contribution of base flow increased with season as total flow decreased. IATC-241 produced high concentrations and high frequencies of detections for SMZ and TYL, but monitoring site was not a significant ($p > 0.05$), SMZ ($p = 0.0621$) and TYL ($p = 0.7204$) contributor to the Tobit regression model. The remaining subsurface sites did not contribute statistical significance to the regression, except for IABC-350 for SMZ.

Even though the tile-drainage sites (IATC241 and IATC242) do not contribute to the Tobit model, the detection of SMZ and TYL demonstrates the ability of tile drains to transport antibiotics from land-applied manures into the subsurface environment, then to surface waters. Furthermore, the increase of baseflow percentage as the season transpires highlights the importance of monitoring subsurface drainage, due to the capability to transport antibiotics. This is consistent with results by Kay et al. (2004), who first demonstrated the transport of antibiotics through tile drains.

3.5. Comparison between POCIS and grab samples

Comparing POCIS results to those for grab samples is difficult due to the duration of the sampling period between the two methods of sampling (Morin et al., 2012). The biggest shortcoming of discrete grab sampling is that it provides only a snapshot or an instantaneous estimate of environmental levels, neglecting episodic events and overestimating concentrations (Thomatou et al., 2011; Vrana et al., n.d.). The POCIS provides time integrative sampling by capturing episodic events, thereby providing a more complete picture. The most noticeable difference observed between sampling methods was detection frequency. ATZ had a detection frequency of 100% for both methods, but SMZ and TYL had higher detection frequencies for POCIS, at 82% and 68% respectively. In comparison, SFIR grab samples detected SMZ at 59% and TYL at 60%. The higher detection frequencies for POCIS samples could be explained by its lower LOD/LOQ compared to that of the grab samples. The POCIS improves the LOD by concentrating sequestered analytes of interest. Estimated POCIS concentrations were lower for SMZ and TYL, compared to grab samples (Fig. 4). A similar relationship was observed by Jones-Lepp et al. (2012).

4. Conclusion

Baseline knowledge on concentrations, occurrence, transport, and temporal behavior of SMZ, TYL, and ATZ in a swine dominated watershed are presented. This study suggests SMZ, TYL, and ATZ were all ubiquitous in SFIR with detection frequencies of 68–100%. We demonstrated application of POCIS to monitor and detect antibiotics at sub-inhibitory concentrations in tile drained landscapes. The detection of SMZ & TYL was higher with POCIS samples than grab samples. The POCIS technology resulted in a lower percentage of censored data for all analytes, compared to grab samples.

While the half-life of these antibiotics are relatively short term, they have shown the ability to be persistent throughout the year in the SFIR, possibly releasing from the terrestrial environment in an episodic nature due to their sources of input. At the single tile drain site monitored by POCIS, IATC-241, a high occurrence of SMZ and TYL was observed across the duration of the study. More importantly, this study verifies the role of tile drainage in the transport TYL in an agricultural watershed. TYL is thought to be less available in the aqueous phase, and more likely sorbed to sediment or soil, suggesting that runoff is the main mechanism for transport.

TWA concentrations for SMZ and TYL were an order of magnitude lower (ng L^{-1}) than the minimum inhibitory concentrations (MIC) for *E.coli*. Still, the prevalence of these antibiotics at sub-inhibitory concentrations could be a cause for concern, due to the potential selective pressure from these antibiotics on the retention of resistance genes (Andersson and Hughes, 2012; Gullberg et al., 2014). The fate and transport of these analytes are impacted by their time of application, hydrological conditions of the watershed, and seasonality. SMZ and ATZ concentrations were found to be statistically significant during growing and harvest seasons, consistent with other studies which indicated similar trends during summer months. By identifying the seasonal fate and occurrence of these analytes, we can be proactive by focusing on the environmental conditions (precipitation, runoff, erosion) and land management techniques (timing of manure application, surface and subsurface drainage) which influence their persistence in the environment, thereby by reducing their potential environmental impact. Management options which have been proven to reduce the transport of antibiotics in the environment, include controlled tile drainage systems (surface water) and vegetative buffer strips (surface runoff).

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Appendix A. Supplementary information

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.08.090.

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Supplementary Information

Monitoring Tylosin and Sulfamethazine in a Tile-Drained Agricultural Watershed Using Polar Organic Chemical Integrative Sampler (POCIS)

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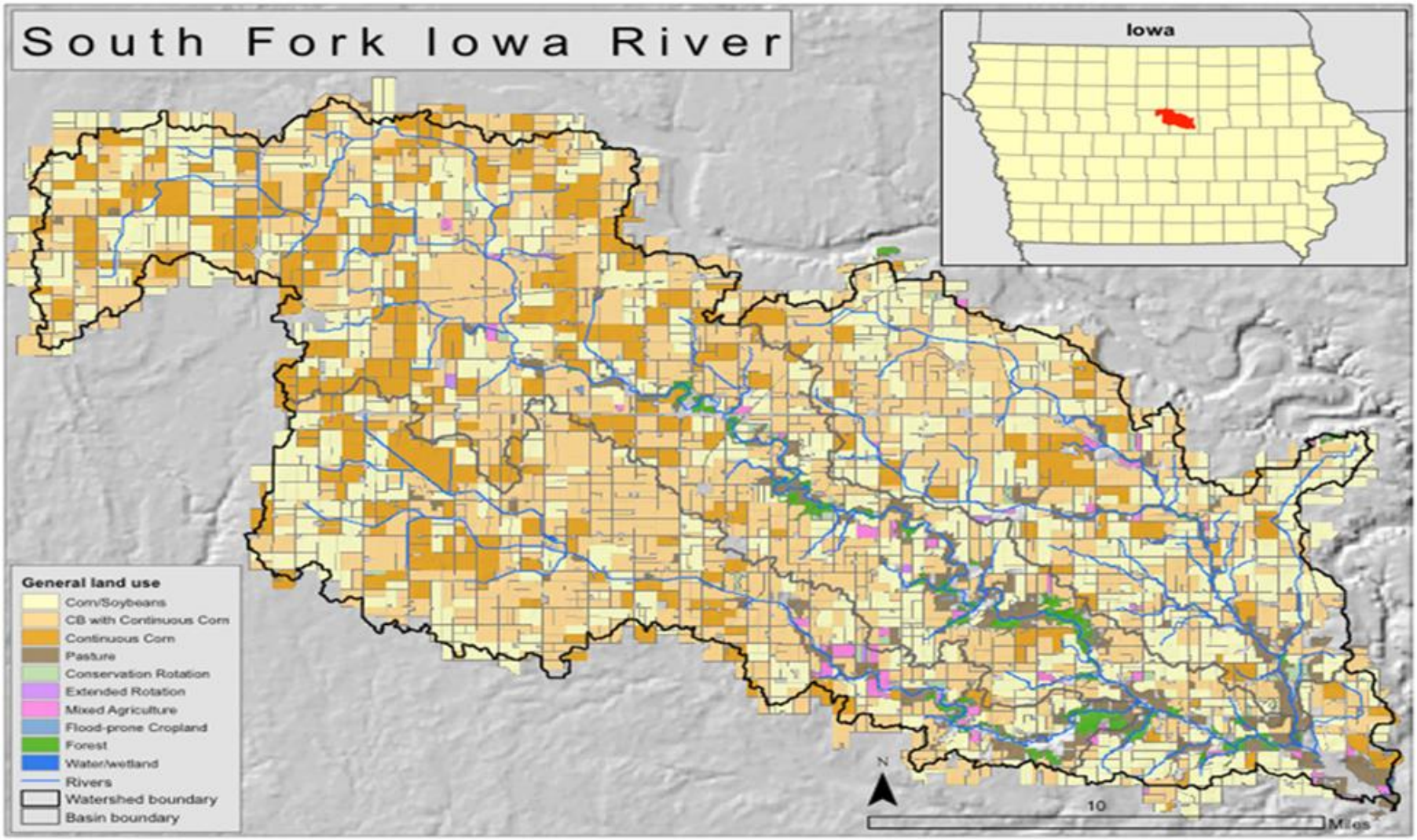
Science of the Total Environment



SI Figure 1. Land use of the South Fork Iowa River watershed of the Iowa River (SFIR). Color shading denotes land use. The map inset shows the extent of the SFIR watershed boundary in central Iowa.

SI Table 1. Sampling sites in the SFIR watershed and their animal unit's (AU's), number of confined animal feeding operations (CAFOs), and sub-basin drainage area.

Site ID	Sub Basin Area (ha)	Animal Unit (AU)	CAFO Count
IATC-241	1,043	0	0*
IATC-242	150	0	0*
IATC-323	17,178	94,916	55
IASF-450	39,798	138,437	91
IABC-350	18,118	25,737	23

*No CAFOs are in the drainage areas of IATC-241 and 242, but swine manure injection occurs (Kevin Cole, USDA-ARS, personal communication)

SI Table 2. Optimized conditions for mass spectrometer quantification.

Compound	Precursor Mass (m/z)	Product Ions (m/z)	Confirmation Ratio	Retention Time	Period
Sulfamethazine	279.1	186		4.35	1
		124.1	51		
		156	32		
Simeton (IS)	198.1	68		4.35	1
Tylosin A	916.5	174.1		6.40	2
		772.4	61		
		88.1	18		
Atrazine	216.1	174		7.20	2
		68	33		
		62	12		

SI Table 3. SFIR watershed POCIS detection frequencies for 2013 – 2015 for sulfamethazine (SMZ), atrazine (ATZ), and tylosin (TYL).

SFIR 2013 POCIS Detection Frequencies

Preplant Season (n = 6)				Growing Season (n = 6)				Harvest Season (n =6)			
Site	SMZ	ATZ	TYL	Site	SMZ	ATZ	TYL	Site	SMZ	ATZ	TYL
241	100	100	100	241	100	100	100	241	100	100	100
323	100	100	100	323	100	100	83.3	323	83.3	100	100
350	100	100	100	350	100	100	66.7	350	83.3	100	100
450	100	100	100	450	100	100	66.7	450	50	100	100

SFIR 2014 POCIS Detection Frequencies

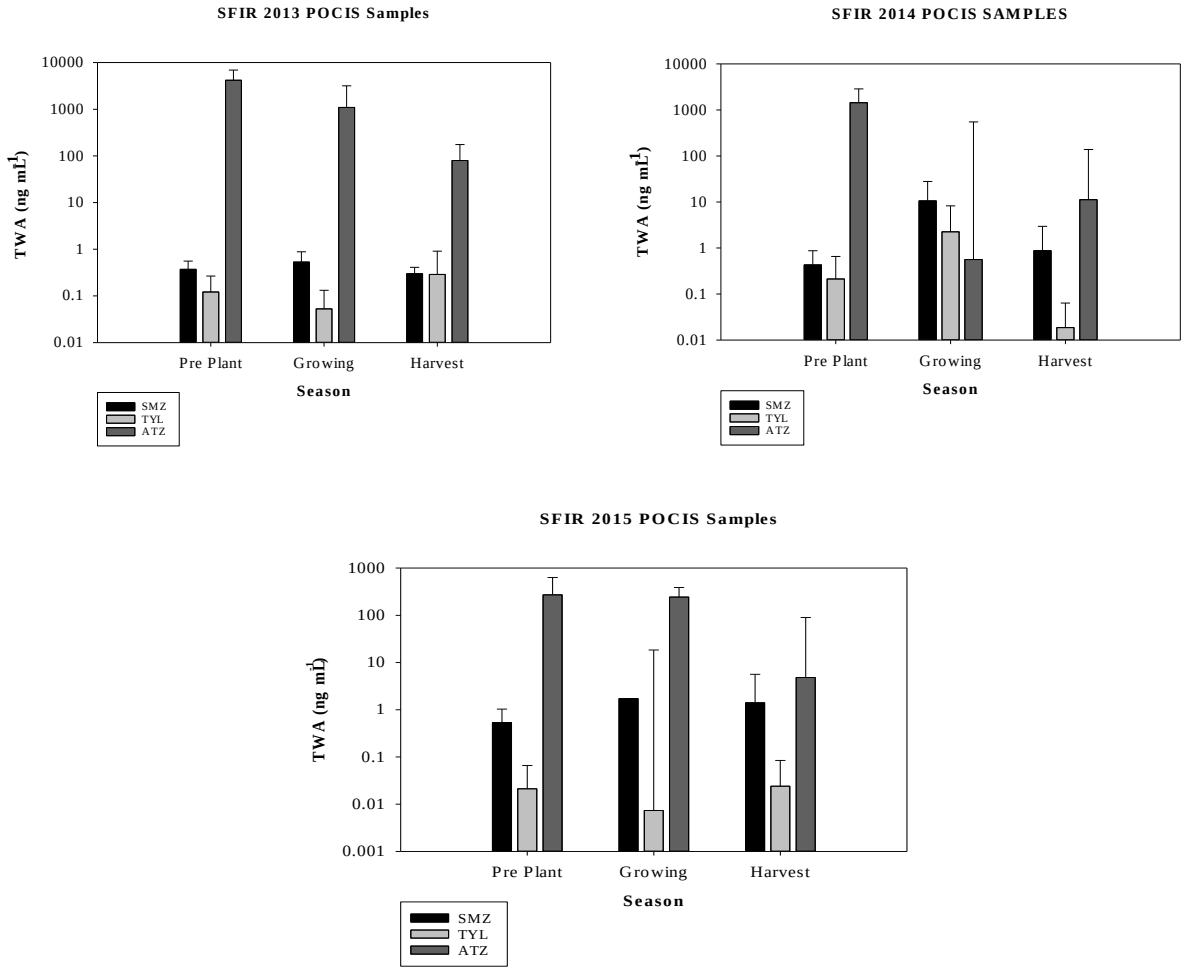
Preplant Season (n = 8)				Growing Season (n = 10)				Harvest Season (n = 10)			
Site	SMZ	ATZ	TYL	Site	SMZ	ATZ	TYL	Site	SMZ	ATZ	TYL
241	100	100	50	241	100	100	100	241	100	100	10
323	75	100	62.5	323	100	100	50	323	83.3333	100	41.6667
350	12.5	100	50	350	50	100	75	350	50	100	16.6667
450	50	100	50	450	90	100	50	450	50	100	8.33333
Detection Frequencies (%)				*241 only 2 reps* Detection Frequencies (%)				Detection Frequencies (%)			

SFIR 2015 POCIS Detection Frequencies

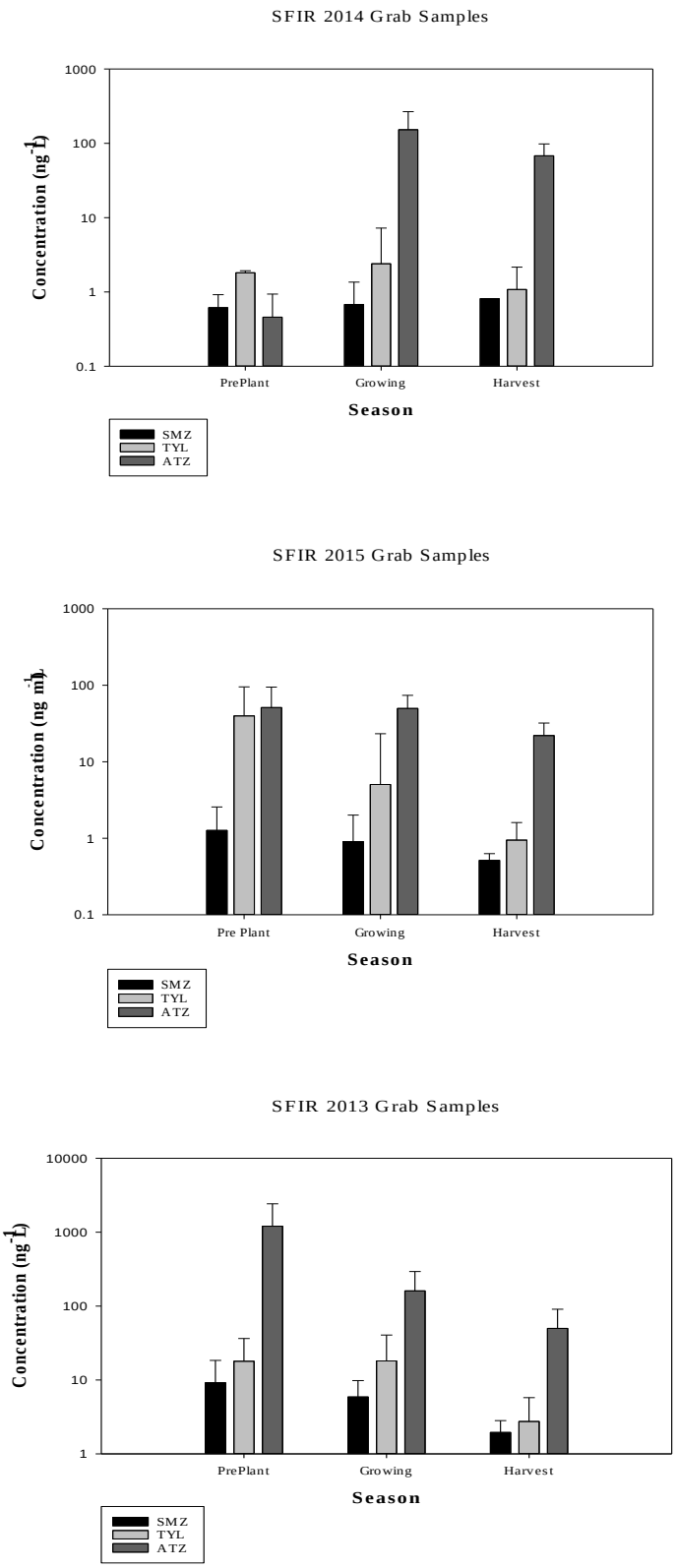
Preplant Season (n = 10)				Growing Season (n = 10)				Harvest Season (n =12)			
Site	SMZ	ATZ	TYL	Site	SMZ	ATZ	TYL	Site	SMZ	ATZ	TYL
241	100	100	90	241	100	100	100	241	100	100	80
323	100	100	50	323	90	100	88.9	323	91.7	100	50.0
350	40	100	60	350	70	100	70.0	350	50	100	58.3
450	70	100	50	450	100	100	60.0	450	100	100	70.0
Detection Frequencies (%)											

SI Table 4. Tobit regression model for POCIS time-weighted average (TWA) concentrations.

Tobit Model POCIS Parameter Estimates						
Parameter	**SMZ Censored at 0.1627 ng L^{-1}**	**P-value**	**TYL Censored at 0.00965 ng L^{-1}**	**P-value**	**ATZ No Censoring**	**P-value**
Intercept	-2.13051	0.0687	-1.183496	0.0193	1346.842198	<.0001
	(1.170291)		(0.505903)		(249.7384)	
Site						
241	2.196570	0.0621	-0.182371	0.7204	-731.383906	**0.0047**
	(1.177351)		(0.509552)		(258.88809)	
323	0.077784	0.9461	-0.146444	0.7653	-125.310228	0.6142
	(1.149740)		(0.490626)		(248.567905)	
350	-4.713769	**0.0002**	-0.694056	0.1691	4.066627	0.987
	(1.251878)		(0.504736)		(250.314315)	
450	0	.	0	.	0	.
	.		.		.	
Year						
2013	-0.725826	0.5348	1.539259	**0.0009**	2227.911049	**<.0001**
	(1.169477)		(0.463497)		(247.060386)	
2014	2.826082	**0.0033**	0.553909	0.1763	478.630123	**0.017**
	(0.961719)		(0.409644)		(200.501239)	
2015	0	.	0	.	0	.
	.		.		.	
Season						
Growing	5.60128	**<.0001**	0.682676	0.1296	-1053.512058	**<.0001**
	(1.122245)		(0.450448)		(234.646882)	
Harvest	0.916164	0.3984	-0.561636	0.2126	-1653.381540	**<.0001**
	(1.084954)		(0.450558)		(222.730018)	
Preplant	0	.	0	.	0	.
	.		.		.	
Sigma	6.853898	**<.0001**	2.642441	**<.0001**	1521.803376	**<.0001**
	(0.328730)		(0.154783)		(63.189924)	
Log Likelihood	-775.54633		-437.78315		2537	
Significance = (P < 0.05)						



SI Figure 2. Seasonal averages of SFIR POCIS TWA concentrations (2013 – 2015). Error bars are presented as the standard deviation of the seasonal mean.



SI Figure 3. Seasonal SFIR grab sample concentrations (2013 – 2015). Error bars are presented as the standard deviation of the seasonal mean.